



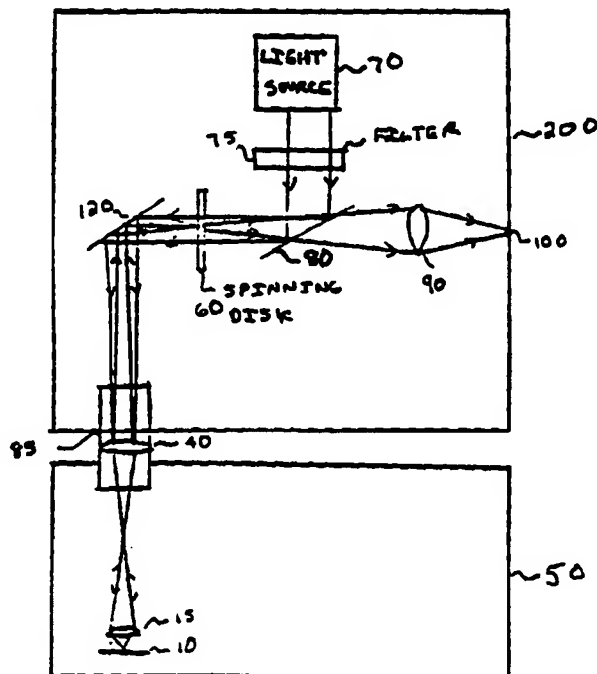
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(21) International Application Number: PCT/US98/22254 (22) International Filing Date: 23 October 1998 (23.10.98) (30) Priority Data: 08/957,454 24 October 1997 (24.10.97) US (71) Applicant (for all designated States except US): ATTO INSTRUMENTS, LLC. [US/US]; 1450 Research Boulevard, Rockville, MD 20850 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): BROOKER, Gary [US/US]; 9212 Bentrige Avenue, Potomac, MD 20854 (US). McDONALD, Scott [US/US]; 19004 Steeple Place, Germantown, MD 20874 (US). ADAMS, Gregory [US/US]; 7704 Lake Drive, Manassas, VA 20111 (US). BROOKER, Jeff [US/US]; 12810 New Parkland Drive, Herndon, VA 22071 (US). (74) Agents: SPIVAK, Marvin, J. et al.; Oblon, Spivak, McClelland, Maier & Neustadt, P.C., Suite 400, 1755 Jefferson Davis Highway, Arlington, VA 22202 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.	

(54) Title: MICROSCOPE ATTACHMENT FOR HIGH PRECISION AND EFFICIENT IMAGING

(57) Abstract

A novel attachment for a microscope to be coupled to an optical coupling tube of a microscope. The attachment for a microscope includes a light source (70) generating a quasi-collimated light output. Elements are provided for reflecting the light output from the light source towards a specimen in the microscope. Further, elements are provided for propagating a reflection of the light output from the light source from the specimen to a viewing point. The elements for achieving the reflecting and the elements for achieving the propagating may each include a spinning Nipkow disk (60) and a dichroic mirror (80). The quasi-collimated light output from the light source directly impinges on the Nipkow disk and without passing through a lens. A right angle mirror can also be positioned as one of the elements for achieving the reflecting and the propagating.



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TITLE OF THE INVENTION**MICROSCOPE ATTACHMENT FOR HIGH
PRECISION AND EFFICIENT IMAGING**

5

BACKGROUND OF THE INVENTION**Field of the Invention:**

The present invention is directed to an attachment for a microscope which imparts improved resolution and which allows a high precision and efficient imaging of a specimen.

10

Discussion of the Background:

Confocal microscopy is established as a technique used in a great number of laboratories. Confocal optical microscopes, and particularly scanning confocal optical microscopes, are known for having an extremely short depth of focus and improved transverse resolution. A confocal optical microscope includes an internal light source to
15 illuminate an object.

Confocal microscopes, however, are very expensive and may have only certain limited applications. Therefore, it would be beneficial to provide a confocal attachment to a standard microscope which would allow confocal microscopy in conjunction with the standard microscope. Such a confocal attachment would have its own internal light
20 source. One example of such a background confocal attachment is the Zeiss CSM shown schematically in Figure 4.

The confocal attachment of Figure 4 connects to a standard optical microscope and includes a tube head 141 as an output viewing point with certain optical elements. The standard optical microscope is shown as elements 161-163 in Figure 4. Element 162

is a tube lens which focuses light onto a specimen 163. Light reflected off the specimen 163 is output to a standard viewing point 161 of the standard microscope.

The confocal attachment of Figure 4 provides a way to illuminate the specimen 163 from light output from a light source 149. The specimen 163 can then be viewed at
5 the output of the tube head 141 or at a television output 145 through a TV selector 147

With this confocal attachment, light is output from the light source 149, which for example may be a halogen or HBO light source. The output light from light source 149 passes through a motorized aperture diaphragm 151 and a filter 153. The output light then continues and passes through a focusing lens 156. The light passing through this
10 focusing lens 156 then impinges on a motorized beam splitter 155 which is designed to reflect the output light from the light source 149 towards a removable spinning disk 157. The lens 156 is chosen such that the light output from the light source 149 focuses onto the removable spinning disk 157. The light focused on the spinning disk 157 then passes through the removable spinning disk 157 to reflect off an autofocus sensor 159 (which is
15 optional). The light then reflects off the autofocus sensor 159 through a tube lens 162 to impinge on a sample 163.

The reflected light off of sample 163, which is an image of the sample 163, then follows a return path through tube lens 162, off the autofocus sensor 159, to be again focused on the spinning disk 157. This reflected light then passes through the motorized
20 beam splitter 155 (beam splitter 155 is designed to pass the reflected excitation light from sample 163, which is at a different wavelength than the emission light output from light source 149), through a further beam splitter 143, and through further optical elements to the tube head 141 where the image of the sample can then be viewed.

This confocal attachment for a standard microscope as shown in Figure 4, however, suffers from some significant drawbacks.

First, this confocal attachment focuses the emission light output from the light source 149 onto the spinning disk 157 through focusing lens 156. This ensures that the emission light is focused on a spinning disk 157, but also results in introducing the focusing lens 156 through which significant optical transmission losses arise.

Further, a drawback with the confocal attachment of Figure 4, and other confocal microscopes, is chromatic aberration. In confocal microscopy, since sub-micron dimensions are being imaged, chromatic aberration (i.e. the diffraction of the color components of a light source by different amounts) becomes a significant problem. Chromatic aberration is introduced any time that a light source is focused. In the confocal attachment of Figure 4, light from the light source 149 is focused by both focusing lens 156 and tube lens 162. As a result, two instances of introducing chromatic aberration result in the confocal attachment of Figure 4. Correcting such chromatic aberration requires rendering the optics in the confocal attachment of Figure 4 more complicated.

Further, the confocal attachment of Figure 4 includes a complicated and lengthy optical path including several optical elements provided for propagating the light generated from the light source 149 and propagating the light reflected off the sample 163. The use of such a large number of optical elements results in great optical transmission loss in the imaging of the sample.

SUMMARY OF THE INVENTION

Accordingly, one object of the present invention is to provide a novel microscope attachment, which may find particular application in a confocal optical microscope, which is simple, small in size and which allows a high precision and efficient imaging of
5 a specimen.

A further object of the present invention is to provide a novel microscope attachment which minimizes the effect of any stray light.

A further object of the present invention is to provide a novel microscope attachment which minimizes the number of optical elements, to thereby reduce any
10 optical transmission loss.

The present invention achieves these noted and other objects by providing a novel attachment for a microscope to be coupled to an optical coupling tube of the microscope. The novel attachment for a microscope of the present invention includes a light source generating a quasi-collimated light output. Elements are provided for reflecting the light
15 output from the light source towards a specimen in the microscope. Further, elements are provided for propagating a reflection of the light output from the light source from the specimen to a viewing point. The elements for achieving the reflecting and the elements for achieving the propagating may each include a spinning Nipkow disk and a dichroic mirror. The quasi-collimated light output from the light source directly impinges on the
20 Nipkow disk, i.e. without being focused on the Nipkow disk and without passing through a lens. A right angle mirror can also be positioned as one of the elements for achieving the reflecting and the propagating.

BRIEF DESCRIPTION OF THE DRAWINGS

A more complete appreciation of the present invention and many of the attendant advantages thereof will be readily obtained as the same becomes better understood by reference to the following detailed description when considered in connection with the
5 accompanying drawings, wherein:

Figure 1 shows a first embodiment of the present invention;

Figure 2 shows a second embodiment of the present invention; and

Figure 3 shows a third embodiment of the present invention; and

Figure 4 shows a background confocal attachment for a standard microscope.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Referring now to the drawings, wherein like reference numerals designate identical or corresponding parts throughout the several views, and more particularly to Figure 1 thereof, a first embodiment of the present invention is shown in schematic detail.

15 The present invention is directed to an attachment 200 for a microscope 50. The microscope 50 can, as one example only, be a Zeiss AXIOVERT 135.

The microscope 50 includes a specimen 10 which is illuminated with light from a light source (not shown) internal to the microscope 50 through an objective lens 15. An image of the specimen 10 then passes through the objective lens 15 to a viewfinder (not
20 shown) of the microscope 50 to view the specimen 10.

The confocal module attachment 200 of the present invention is a confocal module which results in using the standard microscope 50 as a confocal microscope in

instances when confocal microscopy is desired. The microscope 50 can be designed to operate with certain attachments, and thus includes an output to which an optical coupling tube 85 which allows an image of the specimen 10 to be output therefrom can be connected. The confocal module attachment 200 includes the output optical coupling tube 85 to connect to an output of the microscope 50. The optical coupling tube 85 includes a specifically designed lens 40, as discussed in further detail below.

According to a first embodiment of the present invention as shown in Figure 1, the confocal module attachment 200 includes a right angle mirror 120, a spinning disk 60, which may preferably take the form of a Nipkow disk, a dichroic mirror 80, a focusing lens 90, and a viewing point 100. The confocal module attachment 200 of the present invention also includes a light source 70. The light source 70 differs from a light source internal to the microscope 50 to thereby allow different illumination of the specimen 10. This light source 70 may, as one example, take the form of a 100 watt HBO mercury arc lamp. An output of this light source 70 initially passes through a filter 75.

The confocal module attachment 200 of the present invention is particularly adapted to work in fluorescence in which a dye, such as Fura-2 or fluorescein, coats the specimen 10. In such an operation excitation light from the light source 70 differs in wavelength from emission light reflecting off the specimen 10. The filter 75 is selected to ensure a proper wavelength of the excitation light. As a specific example, when Fura-2 is utilized as the dye for the specimen 10, the filter 75 may be chosen to pass only excitation light with a wavelength of 334 nm, and this results in emission light from the specimen 10 having a wavelength greater than 500 nm. If fluorescein is utilized as the

dye for the specimen 10, the filter 75 may be chosen to pass only excitation light with a wavelength of 488 nm, which results in emission light from the specimen 10 having a wavelength greater than 500 nm.

The device of the present invention as shown in Figure 1 operates as follows.

- 5 The light source 70 generates quasi-collimated excitation light which is filtered by filter 75 to let only light of a desired wavelength to pass. The light which passes through the filter 75 then reflects off dichroic mirror 80, passes through the spinning disk 60 and then reflects off the right angle mirror 120. The dichroic mirror 80 is designed to reflect this wavelength of excitation light and to pass the wavelength of reflected emission light.
- 10 The excitation light then passes through the optical coupling tube 85, including the tube lens 40, and objective lens 50 onto the specimen 10. The excitation light then reflects off the specimen 10, to thereby provide an image of the specimen 10 as emission light. The emission light then passes again through the objective lens 15 and through the tube lens 40 of the optical coupling tube connector 85. The emission light reflected from the
- 15 specimen 10 then further proceeds off the right angle mirror 120, through the spinning disk 60, through the dichroic mirror 80 (which again passes light of the emission light wavelength), through the focusing lens 90 and onto the viewing point 100.

- One of the significant features of the present invention is that the light output from the light source 70 is quasi-collimated light and it is this quasi-collimated light
- 20 which passes through the spinning disk 60. Stated another way, the confocal module attachment 200 of the present invention does not focus the light from the light source 70 onto the spinning disk 60, but instead allows quasi-collimated light to pass through the spinning disk 60. As a comparison, the background art of Figure 4 focuses the light from

the light source 149 onto the spinning disk 157 through a focusing lens 156. However, the utilization of such a focusing lens 156 introduces optical losses into the emission light in the background art of Figure 4. In the confocal module attachment 200 of the present invention, instead of focusing light from the light source 70 onto the spinning
5 disk 60, quasi-collimated light passes through the spinning disk 60, to thereby avoid optical transmission losses which would result if the light from the light source 70 was focused onto the spinning disk 60 through a focusing lens.

This provides a further significant benefit in the present invention in that it limits chromatic aberration is limited, thereby allowing simpler optics in the present invention.
10 As discussed above, chromatic aberration is introduced when color components of a light source are diffracted differently through focusing lenses. The background art of Figure 4 introduces chromatic aberration through the focusing lens 156. The confocal microscope attachment 200 of the present invention reduces chromatic aberration by again not including a focusing lens between the light source 70 and the spinning disk 60. By not
15 forming any lenses between the light source 70 and spinning disk 60 chromatic aberration can be minimized in the present invention.

In this way, by not introducing any optical lens elements between the light source 70 and the spinning disk 60, and thereby not focusing an output from the light source 70 onto the spinning disk 60 but conversely allowing quasi-collimated light to pass through
20 the spinning disk 60, optical transmission loss and chromatic aberration can be minimized in the confocal module attachment 200 of the present invention.

Further, in the present invention the positioning and type of the lens 40 and the positioning of the spinning disk 60 are selected so that the excitation light is focused on the specimen 10 and the reflected emission light 15 is focused on the spinning disk 60.

According to a further feature of the present invention, the spinning disk 60 can
5 be slightly offset with respect to the right angle mirror 120. That is, the spinning disk 60 can be positioned such that it is not perpendicular to light reflected from the right angle mirror 120. Such an offset can be very small, for example on the order of $1^\circ 40''$. This feature of the present invention allows a structure such that light reflecting from a backside of the spinning disk 60, i.e., a side closest to the viewing point 100, does not
10 pass through the dichroic mirror 80 and the focusing lens 90 to the viewing point 100, but instead such reflected light is slightly offset from reflecting on the dichroic mirror 80. This light reflecting off the back side of the spinning disk 60 is thus a cast-off light. The spinning disk 60 must also be positioned a certain distance away from the viewing point 60 to allow the cast-off light to not impinge on the viewing point 100. Such cast-off
15 light does not impinge on the viewing point 100 but instead misses the viewing point 100. Similarly, with such an offset of the spinning disk 60 light reflecting off a front side of the spinning disk 60, i.e., a side closest to the right angle mirror 120, does not impinge on the specimen 10.

The spinning disk 60 is structured such that light only selectively passes
20 therethrough. For example the spinning disk 60 may take the form of a solid disk including slits or small holes. For this reason, a preferable form of the spinning disk 60 is a Nipkow disk.

One of the significant benefits in the present invention is also the overall minimization of optical elements. The use of several optical elements in such a device as in the present invention, e.g., as in the background art of Figure 4, would significantly limit the power of the output at the viewing point 100. The present invention has been
5 designed to minimize the number of optical elements, and thus to minimize the optical transmission loss associated with each such optical element, to provide a high light output at the viewing point 100.

As is clear from Figure 1 of the present invention, only one lens, the focusing lens 90, is placed between the spinning disk 60 and the viewing point 100 and no lens or
10 pinhole is positioned between the light source 70 and the spinning disk 60. This minimization of optical elements in the present invention enables achieving high efficiency imaging of sample 10 as discussed above.

The present invention also provides an optical design which works in fluorescence for all dyes which are excited in the ultraviolet range and emit in the visible
15 range, such as Fura-2. Such dyes may typically result in achromatic aberrations and need special corrections with lens systems on both excitation and emission. However, the present invention with its simple optical system does not require special corrections with lenses on both excitation and emission as discussed above.

One possible modification of such a device of the present invention as shown in
20 Figure 1 is shown in Figure 2. The device of Figure 2 is similar to the device of Figure 1 except that the spinning disk 60, which again is preferably in the form of a Nipkow disk, is positioned between the optical coupling tube 85 and the right angle mirror 120, rather than being positioned between the right angle mirror 120 and the dichroic mirror 80 as in

Figure 1. However, the device of Figure 2 has a similar overall operation as discussed above with respect to the device of Figure 1, particular again as quasi-collimated light passes through the spinning disk 60 and as no optical lens elements are formed between the light source 70 and the spinning disk 60.

5 Each of the devices as shown in Figures 1 and 2 have the viewing point 100 at a right angle with respect to the output from the specimen 10 through the tube lens 40. Such a right angle viewing point 100 may often be beneficial from a space point of view in attaching the confocal module attachment 200. For example, the confocal module attachment 200 of Figures 1 and 2 can be placed against a wall. However, a further
10 embodiment of the present invention which even further minimizes optical loss is shown in Figure 3.

 As shown in Figure 3, a further embodiment of the present invention eliminates the right angle mirror 120 and has a simple linear output. Again in such a device as shown in Figure 3 a similar operation as discussed above with respect to the
15 embodiments of Figures 1 and 2 is also possible, and the same benefits therein are achieved.

 Obviously, numerous additional modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the present invention may be practiced
20 otherwise than as specifically described herein.

CLAIMS:

1. An attachment for a microscope, comprising:
a light source generating a quasi-collimated light output;
5 means for reflecting the quasi-collimated light output from the light source
towards a specimen in the microscope;
means for propagating a reflection of the light output from the light source from
the specimen as a reflected image of the specimen to a viewing point.
2. The microscope attachment according to Claim 1, wherein the reflecting
10 means and propagating means each both include a spinning disk and a dichroic mirror.
3. The microscope attachment according to Claim 2, wherein the spinning disk
comprises a Nipkow disk.
4. The microscope attachment according to Claim 1, wherein the propagating
means includes a focusing lens.
- 15 5. The microscope attachment according to Claim 2, wherein the propagating
means further includes a focusing lens formed between the dichroic mirror and the
viewing point.
6. The microscope attachment according to Claim 1, wherein the reflecting
means and propagating means each include a right angle mirror.
- 20 7. The microscope attachment according to Claim 6, wherein the right angle
mirror is positioned between the spinning disk and the coupling tube.
8. The microscope attachment according to Claim 6, wherein the right angle
mirror is positioned between the dichroic mirror and the spinning disk.

9. The microscope attachment according to Claim 2, wherein the propagating means includes only a single focusing lens.

10. The microscope attachment according to Claim 2, wherein the propagating means further includes only a single focusing lens formed between the dichroic mirror
5 and the viewing point.

11. A microscope attachment, comprising:
a light source for outputting quasi-collimated excitation light;
a spinning disk through which the quasi-collimated excitation light passes;
a first focusing lens for focusing the quasi-collimated excitation light passing
10 through the spinning disk onto a specimen to generate an emission image of the specimen
and for focusing the emission image of the specimen onto the spinning disk;
a second focusing lens for focusing the emission image focused onto the spinning
disk onto a viewpoint.

12. The microscope attachment according to Claim 11, further comprising a filter
15 positioned between the light source and the spinning disk to filter the quasi-collimated
excitation light to have a predetermined frequency.

13. The microscope attachment according to Claim 11, wherein the spinning disk comprises a Nipkow disk.

14. The microscope attachment according to Claim 11, further comprising a
20 dichroic mirror formed between the light source and spinning disk.

15. The microscope attachment according to Claim 11, further comprising a right angle mirror formed between the spinning disk and the first focusing lens.

16. The microscope attachment according to Claim 11, further comprising a right angle mirror positioned between the spinning disk and the light source.

17. The microscope attachment according to Claim 14, wherein the spinning disk comprises a Nipkow disk.

5 18. The microscope attachment according to Claim 15, wherein the spinning disk comprises a Nipkow disk.

19. The microscope attachment according to Claim 16, wherein the spinning disk comprises a Nipkow disk.

FIGURE 1

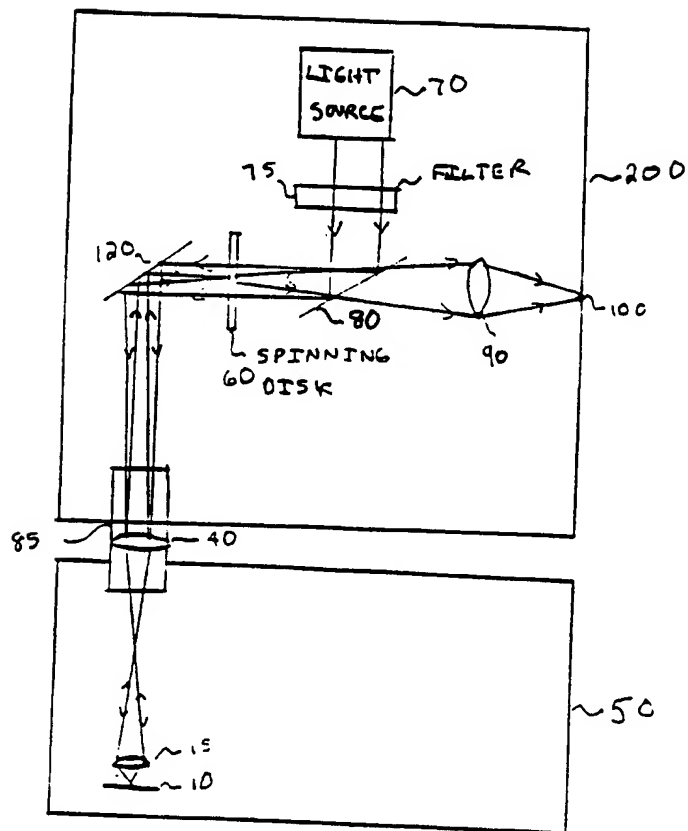


FIGURE 2

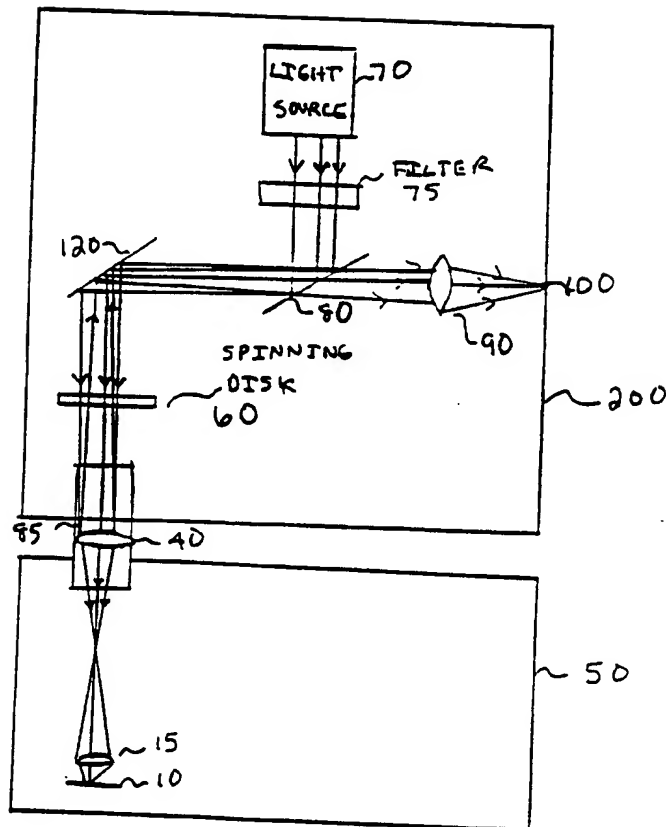


FIGURE 3

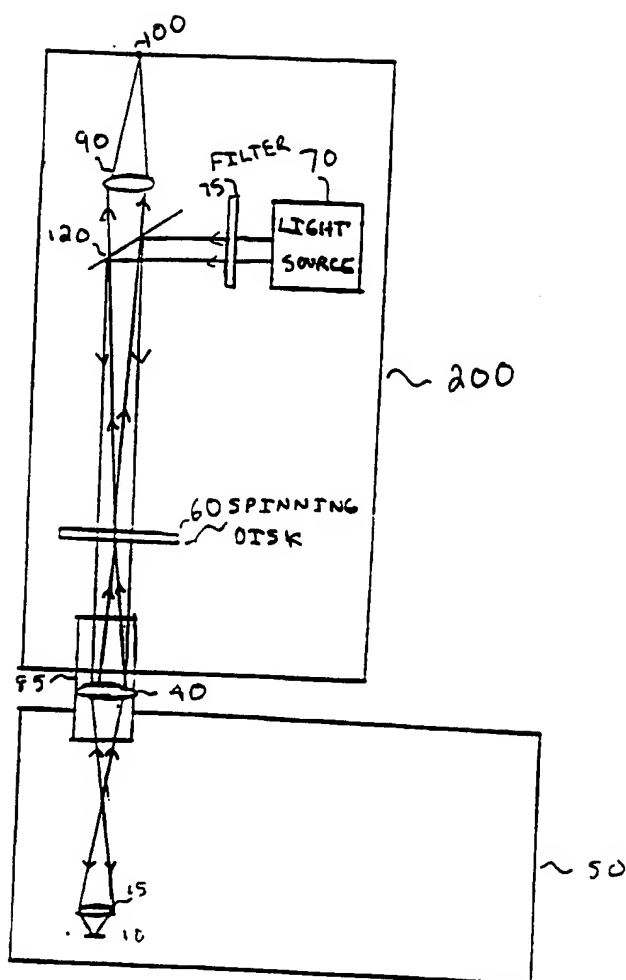
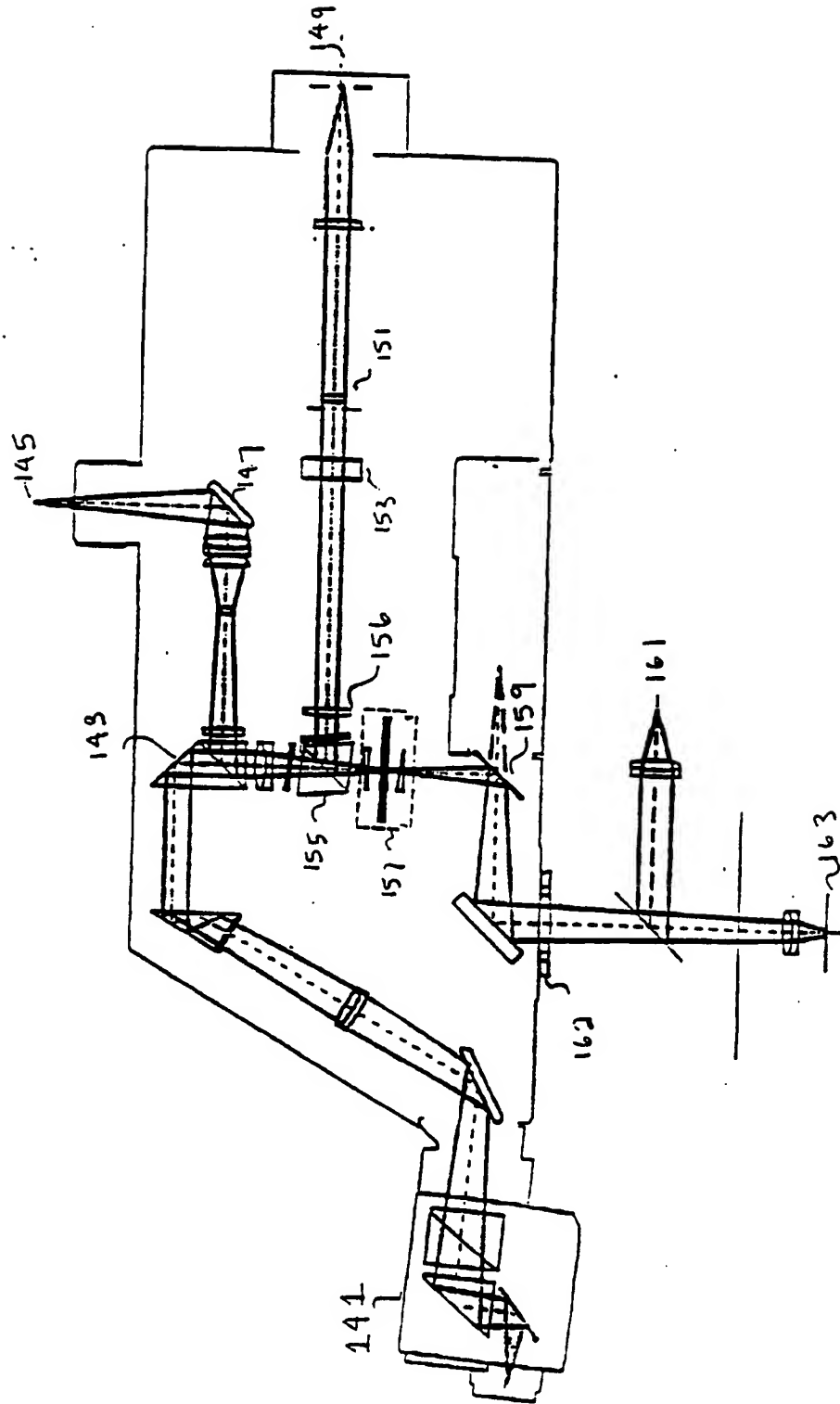


Figure 4
BACKGROUND ART



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/22254

A. CLASSIFICATION OF SUBJECT MATTER IPC(6) :G02B 21/00 US CL :359/368 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 359/368, 385, 389, 234, 235 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,633,751 A (TANAAMI ET AL) 27 May 1997 (27/05/97), see entire document, especially figures 1, 13, and 18.	1-19
A	US 5,235,457 A (LICHTMAN ET AL) 10 August 1993 (10/08/93), see entire document.	1-19
A	US 5,701,197 A (YANO) 23 December 1997 (23/12/97), see entire document.	1-19
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